PRIMER FOR DETECTION OF HUMAN PAPILLOMAVIRUS

Technical Field

The present invention relates to primers specific to the genome of human papillomavirus (hereinafter, referred to as "HPV"), a kit for detecting the HPV genome comprising the primers, and a method of detecting the HPV genome using the primers.

Background Art

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HPV is a double-stranded DNA virus whose circular genome is approximately 8 kb long. HPV inhabits the vagina, and the infection thereof is hard to treat and is not easily made to disappear. HPV infects the epithelial cells of other mammals as well as humans, and generally induces warts, and sometimes malignant tumors, at the site of infection. HPV is detected in over 90% of condyloma accuminata cases (enlarged warts having a cauliflower-like appearance around the genitals or the anus) and almost 100% of cervical cancer cases. In particular, cervical cancer accounts for 22.1% of all cancers found in women in Korea, and is the second leading cause of cancer death among women.

Thus, establishing a method of effectively detecting HPV, which causes cervical cancer, is important for the

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diagnosis, prophylaxis and therapy of the disease. Also, HPV needs to be effectively detected to evaluate the efficacy and toxicity of a vaccine against HPV after vaccination.

nucleic acid-based test for diagnosing infectious disease employs a standard method of isolating nucleic acids from individuals and clinical materials. Since target DNA or RNA is present in clinical specimens in small amounts, several major techniques used in diagnostic laboratories are based on signal amplification and target amplification. These methods aid detection, are useful in the identification of individuals without culture, and contribute to the treatment as well as diagnosis of PCR, which is а infectious diseases. nucleic acid amplification technique (NAT), is widely used because it enables the selective amplification of specific targets, present in low concentrations, to detectable levels. addition the qualitative detection of to viruses, quantitative determination of viral load in specimens is now realized to be of great importance with respect to the diagnosis, prognosis, and therapeutic monitoring of HPV infection (Pfaller M.A, Emer. Infect. Dis. 7, 2, 2001).

The genome of all types of HPV is divided into two major regions: early and late regions. The early region of about 4.5 kb codes for genes which are associated with

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functions including viral DNA replication (E1), induction or suppression of the action of DNA encoding a protein inducing malignant transformation of host cells (E2), synthesis of proteins responsible for the growth of host cells and viruses (E4), stimulation of the activity of epidermal growth factor (EGF) and colony stimulator factor (CSF) receptors (E5), and malignant transformation through permanent survival of cells, activation of oncogenes and inactivation of tumor suppressor genes (E7). In particular, the oncogenic E6 and E7 proteins, which are expressed after. HPV infects the epithelial cells of a host, bind to tumor suppressor proteins of host cells, p53 and respectively, thereby inhibiting the function of the tumor suppressor proteins, leading to the transformation of infected cells, resulting in the development of tumors. The late region of 2.5 kb comprises genes coding for viral major (L1) and minor (L2) capsid proteins and a non-coding region of 1 kb, which is called the long control region (LCR) that regulates the transcription and translation of the two late genes.

With recent rapid advances in molecular biological techniques, the genetic structure of HPV has been identified, revealing genomic sequences of many genotypes of HPV. HPV is classified according to the difference in DNA sequences of E6, E7 and L1 open reading frames (ORFs). When the nucleotide sequences of the ORFs differ by more

than 10%, an HPV is assigned a new genotype. HPV subtypes differ by 2% to 10%, and HPV variants differ by less than 2%.

In order to specifically detect high risk HPV types 16, 18 and 31 and a low risk HPV type 11 among a large number of HPV genotypes, which are detected in tissues of cervical cancer and carcinoma in situ, respectively, the present inventors intended to detect a gene specific to each genotype of these viruses, and selected the L1 gene as such a gene.

In order to specifically detect the HPV L1 gene, the present inventors determined the sequences of L1 genes of the HPV types 11, 16, 18 and 31, which are specifically found in Koreans, and constructed primers capable of specifically binding to the L1 gene of each HPV type. The present inventors found that when PCR was performed with the primers, each HPV genotype can be specifically detected and can be precisely quantified down to very low amounts, thereby leading to the present invention.

20 Disclosure of the Invention

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It is therefore an object of the present invention to provide a primer pair selected from among pairs of primers capable of complementarily binding to the genome of human papillomavirus (HPV) and having nucleotide sequences

represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

It is another object of the present invention to provide a method of detecting the HPV genome, which is based on performing a polymerase chain reaction (PCR) for DNA contained in a biological sample using one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

It is a further object of the present invention to provide a kit for detecting the HPV genome, comprising one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

Brief Description of the Drawings

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The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 schematically represents the construction of

recombinant plasmids constructed with amplified L1 genes of HPV genotypes 11, 16, 18 and 31 (A), and also shows the results of restriction enzyme mapping of the recombinant plasmids (B);

Fig. 2 is an alignment of HPV 16 L1 sequences;

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- Fig. 3 is an alignment of HPV 31 L1 sequences;
- Fig. 4 is an alignment of HPV 11 L1 sequences;
- Fig. 5 is an alignment of HPV 18 L1 sequences;
- Fig. 6 is a multiple alignment of nucleotide

 10 sequences of L1 genes of HPV genotypes 11, 16, 18 and 31

 and the consensus L1 sequence;
 - Fig. 7 shows the results of a sensitivity test using plasmid DNA templates, each of which carry the HPV 11, 16, 18 or 31 L1 gene;
- Fig. 8 shows the results of a differentiality test using plasmid DNA templates, each of which carries the HPV 11, 16, 18 or 31 L1 gene;
 - Fig. 9 shows the results of heat stability and longterm preservation tests for HPV L1 plasmids after storage for 3 weeks;
 - Figs. 10 and 11 show the results of heat stability and long-term preservation tests for HPV L1 plasmids after storage for 15 weeks; and
- Fig. 12 shows the results of an applicability test
 using predetermined amounts of HPV L1 plasmids supplemented
 with various DNA backgrounds.

Best Mode for Carrying Out the Invention

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In one aspect, the present invention relates to primers capable of complementarily binding to the HPV genome.

In a detailed aspect, the present invention relates to a primer pair selected from among pairs of primers capable of complementarily binding to the HPV genome and having the nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

The term "primer", as used herein, refers to a short nucleic acid sequence having a free 3' hydroxyl group, which able to undergo base-pairing interaction with a complementary template and serves as a starting point for replicating the template strand: A primer is able to initiate DNA synthesis in the presence of a reagent for polymerization and four different nucleoside triphosphates in suitable buffers and at a suitable temperature. With respect to the objects of the present invention, primers specifically amplify a specific region of the L1 gene of HPV 16, described in Fig. 2, HPV 31, described in Fig. 3, HPV 11, described in Fig. 4, and HPV 18, described in Fig. 5. Thus, the primers of the present invention consist of a pair of sense and antisense primers having a sequence of 7 nucleotides, and more preferably 10 50

nucleotides, the sequence capable of complementarily binding to the aforementioned HPV L1 gene. In detail, a specific region of the HPV L1 gene may be specifically amplified with a pair of primers having the nucleotide sequences of SEQ ID Nos. 1 and 2 for HPV 11, a pair of primers having the nucleotide sequences of SEQ ID Nos. 3 and 4 for HPV 16, a pair of primers having the nucleotide sequences of SEQ ID Nos. 5 and 6 for HPV 18, and a pair of primers having the nucleotide sequences of SEQ ID Nos. 7 and 8 for HPV 31.

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The primers of the present invention may chemically synthesized using a phosphoramidite support method or other widely known methods. These nucleic acid sequences may also be modified using any means known in the art. Non-limiting examples of such modifications include methylation, capsulation, replacement of one or more native nucleotides with analogues thereof, and internucleotide modifications, for example, modifications to conjugates (e.g., methyl phosphonate, uncharged phosphotriester, phosphoroamidate, carbamate, etc:) phosphorothioate, charged conjugates (e.g., phosphorodithioate, etc.). Nucleic acids may contain one or more additionally covalent-bonded residues, which are exemplified by proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, intercalating agents (e.g., acridine, psoralene, etc.),

chelating agents (e.g., metals, radioactive metals, iron, oxidative metals, etc.), and alkylating agents. The nucleic acid sequences of the present invention may also be altered using a label capable of directly or indirectly supplying a detectable signal. Examples of such a label include radioisotopes, fluorescent molecules, and biotin.

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When PCR was performed with the primers provided in the present invention, which have the nucleotide sequences of SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8, the primers were found to be able to specifically detect each of the four different HPV genotypes and to be sensitive enough to amplify as few as 62.5 copies of a plasmid.

Thus, the present primers may be useful in the detection of HPV infections, the identification of infective HPV genotypes, the epidemiological evaluation of HPV, the effectiveness and toxicity of developed HPV vaccines, and the like.

In another aspect, the present invention provides a method of detecting the HPV genome, which is based on performing a polymerase chain reaction (PCR) for DNA contained in a biological sample using one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having the nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7

and 8.

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The term "biological sample", as used herein, includes, but is not limited to, samples, such as tissues, cells, whole blood, sera, plasma, saliva, sputa, cerebrospinal fluid, urine, or the like, of individuals infected with HPV or suspected of being infected with HPV, or individuals vaccinated with a HPV vaccine.

A method for identifying the presence and genotype of HPV is particularly not limited as long as it employs the aforementioned primers. Examples of such methods include direct identification of HPV DNA using a primer of a specific strand as a probe, Southern blotting, dot blotting, and filter in situ hybridization (FISH). Alternative methods include a method based on amplifying HPV DNA using a pair of primers, genotype-specific polymerase chain reaction (PCR), and general-primer PCR. PCR is most preferred.

The term "polymerase chain reaction (PCR)", as used herein, is a representative nucleic acid amplification technique (NAT), which enzymatically amplifies a specific DNA region of interest in vitro. The PCR method, which was developed in 1985 by Mullis et al., can amplify any segment of a DNA molecule if its boundary sequences are known. PCR basically consists of three major steps: denaturation, annealing and extension. A specific DNA sequence is amplified while these three steps are repeated. In the

first step (denaturation) of PCR, a double-stranded template DNA is denatured into two single strands. In the second step (annealing), primers anneal with the two kinds of single-stranded DNA, in which a sequence desired to be amplified is interposed between the primer binding regions. In the third step (extension), a heat-resistant DNA polymerase extends the primers and synthesizes the complementary strand of the target sequence. This cycle is repeated 25 to 30 times.

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Primers are the most important factor determining the reliability of PCR results. Some primer sequences can give rise to non-specific amplification, leading to false results. In this regard, the present invention provides reliable primer pairs. The performance of PCR with the primer pairs of the present invention enables accurate detection of HPV genotypes and sensitive quantitative analysis of very small amounts. Also, when PCR is carried out with the primer pairs of the present invention, consistent results are obtained upon repeated PCR performance. That is, since the primer pairs of the present invention are highly valid and reliable, the results obtained with the present primer pairs are highly reliable.

In a preferred aspect, the present invention provides a method of detecting the HPV 11 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 1 and 2.

In another preferred aspect, the present invention provides a method of detecting the HPV 16 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 3 and 4.

In a further preferred aspect, the present invention provides a method of detecting the HPV 18 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 5 and 6.

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In yet another preferred aspect, the present invention provides a method of detecting the HPV 31 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 7 and 8.

A PCR for amplifying an HPV gene, in detail the L1 gene, using the primers of the present invention may be carried out through an ordinary PCR method. Also, conditions including time, temperature and cycle number, under which denaturation, annealing and extension reactions are allowed to occur, may vary. In the present invention, PCR conditions included 35 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 1 min or 1 min 30 sec.

In a further aspect, the present invention provides a kit for detecting the HPV genome, comprising one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having the nucleotide sequences represented by SEQ ID Nos. 1 and 2,

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SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

In addition to the primer pairs, the detection kit of the present invention is composed of one or more different compositions, solutions or instruments, which are suitable for analysis methods. Preferably, the kit of the present invention includes the following constituents: a container containing detection primers; amplification reaction tubes or other suitable containers; reaction buffer (pH and magnesium concentration of which may vary); dNTPs; an enzyme such as Taq-polymerase; RNase; and sterile water. More preferably, the kit may further include a plasmid carrying an HPV gene as a positive control in order to realize quantitative analysis. Such a plasmid may be one or more selected from among pGEM-HPV11 L1, pGEM-HPV16 L1, pGEM-HPV18 L1, and pGEM-HPV31 L1, which will be described in the following examples.

A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

EXAMPLE 1: Construction of recombinant HPV L1 plasmids (standard DNA)

PCR primers specific to low risk HPV 11 and high risk

HPV 16, 18 and 31 were designed based on major protein (HPV L1) sequences of the different genotypes of HPV, which are deposited in GenBank. In order to obtain HPV genotypes commonly found in Korean, tissues of Korean cervical cancer patients were obtained from clinical hospitals, and genomic DNA as an HPV genome source was extracted from the tissues. Biological tissue samples were paraffin sections or biopsy samples prepared for pathological examination. PCR was carried out using the extracted genomic DNA with primers having the nucleotide sequences of SEQ ID Nos. 9 to 16, which are listed in Table 1, below. As a result, PCR products of about 1.6 kb were obtained.

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TABLE 1
Primer sequences against the HPV L1 gene for the preparation of national (Korean) standard DNA

HPV genotype	PCR fragment length		PCR primer sequence
HPV 16	1596 bp	Sense	5'-GCCCCCAAGCTTGCCGCCACCATGCAGGTGACTTTTATTTA
111 10	1330 Dp	Anti-	5'-ATCGGGCTCGAGCAGCTTACGTTTTTGCGTTTAGC-3' (SEQ ID
		sense	No. 10)
		Sense	5'-GCCCCAAGCTTGCCGCCACCATGTGCCTGTATACACGG-3' (SEQ
HPV 18	1707 bp	Dense	ID No. 11)
nev 10	1707 DD	Anti-	5'-ATCGGGGAATTCCTTCCTGGCACGTACACGCACACG-3' (SEQ ID
		sense	No. 12)
		Sense	5'-GCCCCAAGCTTGCCGCCACCATGTCTCTGTGGCGGCCTAGC-3'
HPV 31	1515 bp	pelise	(SEQ ID No. 13)
NEA 21	1313 pp	Anti-	5'-ATCGGGGAATTCCTTTTTAGTTTTTTTACGTTTTGCTGGTGTAGTGG-
		sense	3' (SEQ ID No. 14)
		Sense	5'-GCCCCCAAGCTTGCCGCCACCATGTGGCGGCCTAGCGACAGC-3'
HPV 11	1506 bp	Sense	(SEQ ID No. 15)
nev 11	1300 00	Anti-	5'-ATCGGGGAATTCCTTTTTGGTTTTGGTACGTTTTCGTTTGGG-3'
		sense	(SEQ ID No. 16)

PCR was carried out under the following conditions. PCR was carried out using the DNA samples extracted from tissues from patients as templates with 2.5 mM dNTP, reaction buffer, primer pairs (20 pmol) listed in Table 1, According to the optimal annealing and SuperTag Plus. temperature of primers, a cycle of denaturation at 94°C for 1 min, annealing at Ta for 1 min, and extension at 72°C for 1 min 30 sec was repeated thirty five times, followed by final extension at 72°C for 10 min. Each PCR product was pGEM-T-Easy vector (Promega, USA) Plasmid DNA was then transformed into E. coli DH5 α . isolated and digested with EcoRI to determine whether the PCR product was successfully inserted (Fig. 1).

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The nucleotide sequences of the PCR products were determined and compared with previously known nucleotide sequences coding for the L1 protein of HPV genotypes. HPV 16 was compared with AF402678, HPV 31 with J04353, HPV 11 with NC_001525, and HPV 18 with NC_001357. As a result, the nucleotide sequences coding for the L1 protein of the four HPV genotypes, which were identified according to the procedure described above, were found to be highly similar to the conventionally known nucleotide sequences coding for L1 proteins of the HPV genotypes.

EXAMPLE 2: Large preparation and quantification of the recombinant HPV L1 plasmids

E. coli cells transformed with the recombinant HPV L1 plasmids prepared in Example 1 were inoculated in 10 ml of LB medium supplemented with ampicillin, and grown in a shaking incubator at 37°C overnight. Plasmid DNA was then isolated using an alkaline lysis method, and precisely quantified using a spectrophotometer. The plasmid copy number was calculated according to Equation 1, below.

[Equation 1]

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Copy number of 1 kb fragment = (1000 bp \times 660 g/mole) 10 / (6.023 \times 10²³ molecules) = 1 \times 10⁻¹⁸ g (1 fg)

Equation 1 means the following. One copy of a 1 kb (1000 bp) plasmid weighs 1×10^{-18} g (1 fg), and one gram of 1 kb plasmid DNA contains 10^{18} copies of the plasmid.

Using Equation 1, the number of copies of each of different HPV genotype plasmids was calculated, and a plasmid solution having 2000 copies was serially diluted two-fold, thereby yielding $10-\mu l$ solutions containing 2000, 1000, 500, 250, 125 and 62.5 copies of the plasmids.

EXAMPLE 3: Evaluation of the sensitivity of specific primers using the recombinant HPV L1 plasmids

Using the 10 μ l plasmid solutions containing 2000,

1000, 500, 250, 125 and 62.5 copies, prepared in Example 2, PCR was carried out. As a result, PCR primers displayed sensitivity in a manner dependent on the number of plasmid copies (indicating that the 10 μ l DNA solutions respectively contained 2000, 1000, 500, 250, 125 and 62.5 copies of the plasmids). Referring to Fig. 6, PCR primers represented by SEQ ID Nos. 1 to 8 (Table 2) were determined.

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TABLE 2
Primers for detecting the HPV L1 gene

Genotype	Sense primer	Anti-sense primer
HPV 11	TTAGGCGTTGGTGTTAGTGG (SEQ ID No. 1)	AAAATTCATAGCACCAAAGC (SEQ ID No. 2)
HPV 16	TTAGGTGTGGGCATTAGTGG (SEQ ID No. 3)	AAAGTCCATAGCACCAAAGC (SEQ ID No. 4)
HPV 18	TTAGGTGTTGGCCTTAGTGG (SEQ ID No. 5)	AAAGTCCATGGCACCATATC (SEQ ID No. 6)
HPV 31	TTAGGTGTAGGTATTAGTGG (SEQ ID No. 7)	AAAATCCATAGCTCCAAAGC (SEQ ID No. 8)

PCR was carried out as follows. 5 μ l of 2.5 mM dNTP was mixed with 5 μ l of 10 × buffer, primers (20 pmol) of SEQ ID Nos. 1 to 8, 0.5 μ l of Taq polymerase, and distilled water to give a final volume of 40 μ l. The mixture was supplemented with 10 μ l of each template, thereby yielding a PCR mixture. PCR conditions included 35 cycles of denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 10 min. PCR products were then separated on a 1.5% agarose gel for 40 min, and stained with ethicium bromide (EtBr). Band intensity was measured

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using the software, Quantity One (Bio-Rad). regression function was derived in order to determine the relationship between band intensity and the number of plasmid copies, and a relative coefficient R was calculated to determine whether it was greater than 0.9. As a result of the sensitivity test for the PCR amplification method, the band intensity, as shown in Fig. 7, decreased in all of the four HPV genotypes in a manner dependent on the number of plasmid copies, and this PCR method was found to have a sensitivity detecting as few as 62.5 copies of the HPV L1 plasmids. When DNA was run on an agarose gel to determine the relationship between band intensity and the number of plasmid copies, the relative coefficient, as shown in Fig. 7, was greater than 0.9. These results indicate that a plasmid copy number test using the method of the present invention provides reliable results.

EXAMPLE 4: Evaluation of the specificity of primers using the recombinant HPV L1 plasmids

In order to determine whether the primers used in Example 3 specifically amplify each HPV genotype, the primer sets to the four different HPV genotypes were evaluated for whether they differentially amplify L1 templates of different HPV genotypes. PCR was carried out under the same conditions as in Example 3 except that the

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templates were used in a concentration of 1000 copies. PCR was performed with each primer set using each of the four different HPV genotypes as a template. As a result of the PCR with each primer set using 1000 copies of each HPV genotype as a template, all primer sets to HPV 11, 16, 18 and 31 were found to specifically amplify only their corresponding templates (Fig. 8). The results, specifically that the primers of the present invention precisely detect only their corresponding HPV genotypes under optimized PCR conditions, indicate that the different primer sets enable HPV genotyping of clinical samples and differential detection of high risk HPV types 11, 16 and 18, infections of which are most likely to progress to cervical cancer. Thus, the present primers may become a very useful means of clinical diagnosis.

EXAMPLE 5: Evaluation of the heat stability and long-term preservation of the recombinant HPV L1 plasmids

To evaluate the heat stability and long-term preservation of primers, first, 30 μ l of 1000 copies of each HPV L1 plasmid was aliquotted into fifteen DNase/RNase-free vials. The containers were stored at 4°C, 22°C and 37°C for a heat stability test, and at -80°C for long-term storage. Every three weeks, one vial at each storage temperature was subjected to a sensitivity test, which was carried out

according to the same procedure as in Example 3. The PCR with plasmids stored at 22° C and 37° C for three weeks showed negative results, indicating that standard DNA has a very low stability when stored at 22° C and 37° C (Fig. 9). In contrast, standard DNA stored at 4° C and -80° C for 15 weeks still provided highly sensitive PCR results, indicating that the standard DNA of the present invention is stable when stored at -80° C for a long period of time (Figs. 10 and 11).

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10 EXAMPLE 6: Evaluation of the applicability of primers using the recombinant HPV L1 plasmids

The procedure with the HPV L1 templates and primers in the present invention was evaluated to determine whether it could detect the HPV genome in human clinical samples, as follows. Genomic DNA was extracted from human rhabdomyosarcoma (RD), HeLa and SLK cells using genomic DNA extraction kit (Qiagen), the determined using concentration thereof was spectrophotometer. Each DNA solution was diluted to concentrations of 10 ng and 100 ng. 100 μ l of each dilution was aliquotted and stored at $-20\,\mathrm{C}$. Then, PCR was carried out with 100 ng and 1 $\mu \mathrm{g}$ of genomic DNA background using the same templates as in the sensitivity test of Example 3. PCR products were analyzed according to the same method used

for the sensitivity test. In the PCR with the genomic DNA background, the HPV templates, as shown in Fig. 12, could be detected even with as few as 62.5 copies, as in the absence of human genomic DNA. The PCR with genomic DNA background from HeLa cells, which contain the HPV 18 genome, exhibited positive results in all lanes.

Industrial Applicability

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As described hereinbefore, the primer pairs specific to the L1 gene of HPV 11, 16, 18 and 31 according to the present invention may be useful in the detection of HPV infections, the identification of infected HPV genotypes, evaluation of the effectiveness and toxicity of developed HPV vaccines, and the like.

Claims

1. A primer pair selected from among pairs of primers capable of complementarily binding to human papillomavirus (HPV) genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

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- 2. A kit for detecting human papillomavirus (HPV) genome, comprising one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.
- 3. A method of detecting human papillomavirus (HPV) genome, comprising performing a polymerase chain reaction for genomic DNA contained in a biological sample using one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.
- 4. The method as set forth in claim 3, wherein an HPV 11 L1 gene is detected using the primer pair having the

nucleotide sequences represented by SEQ ID Nos. 1 and 2.

5. The method as set forth in claim 3, wherein an HPV 16 L1 gene is detected using the primer pair having the nucleotide sequences represented by SEQ ID Nos. 3 and 4.

6. The method as set forth in claim 3, wherein an HPV 18 L1 gene is detected using the primer pair having the nucleotide sequences represented by SEQ ID Nos. 5 and 6.

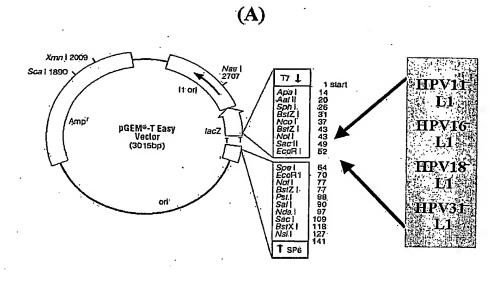
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- 7. The method as set forth in claim 3, wherein an HPV 31 L1 gene is detected using the primer pair having the nucleotide sequences represented by SEQ ID Nos. 7 and 8.
- 8. The kit for detecting the HPV genome as set forth in claim 2, further comprising one or more plasmids selected from among pGEM-HPV11 L1, pGEM-HPV16 L1, pGEM-HPV18 L1 and pGEM-HPV31 L1 as a positive control in order to realize quantitative analysis.

Fig. 1



(B)

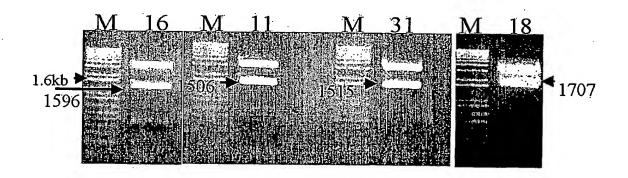


Fig. 2

	1	10	20	30	40	50	60	70	B0	50	100	110	120	130
EF402578 EPV15 Consensus									TTTCAGNISTC TTTCAGNISTC					
EF402678	131	140	150	160	170	180	190	200	210	220	. 830	240	250	260
EPV16 Consensus									INCTIGENSTI INCTIGENSTI INCTIGENSTI					
	261	270	280	230	300	310	320	330	340	350	350	370	380	390
RF402678 HPV16 Consensus									TOCTORCACCT TOCTORCACCT TOCTORCACCT					
	391	400	410	420	430	440	450	450	470	480	430	500	510	520
RF402678 HPV15 Consensus									CREARANTEC CREARANTEC CREARANTEC					
	521	530	540	550	580	570	580	530	600	610	620	530	640	650
RF402678 HPV16 Consensus									ATCCCCATET ATCCCCATET ATCCCCATET					
	651	660	670	580	630	700	710	720	730	740	750	750	770	780
RF402E7B RPV16 Consensus									ANCARANGTO ANCARARGTO INCAMANGTO					
	781	780	B00	810 ·	820	B30	840	850	860	870	880	830	900	910
RF402678 HPV16 Consensus									ATTINTINA ATTINTINA ATTINTINA					
	911	920	930	940	950	360	970	380	990	1000	1010	1020	1030	1040
RF402678 HPV16 Consensus									TGATGCCCRRI TGATGCCCRRI TGATGCCCRRI					
•	1041	1050	1060	1070	10B0	1090	1100	1110	1120	1130	1140	1150	1160	1170
RF402678 RPV16 Consensus									GETGECATATI GETGECATATI GETGECATATI					
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1250	1270	1280	1290 .	1300
RF402578 HPV16 Concensus									COTRENTACO COTRENTACO COTRENTACO					
	1301	1310	1320	1330	1340	1350	1350	1370	1380	1390	1400	1410	1420	1430
107716	CICCO	356855	CETTORAS	TRETTOTOG	STITETERE	TECENSEEN	TILLITETES	เกตอกเกราก	RCCTCCRGCRC RCCTCCRGCRC RCCTCCRGCRC	TTOODCORC	TECETTOR	ODGTOCOCYT	TITECCORET	DOGTTT
		1440	1450	1450	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
HPV16	10005680	umiliic	TECHECOCCT	RENTENGITTI	CCTTIRGERC	ECHOUITITY I	RETREBUCES	CONTRACT	GECRARECTAR GECRARECTAR GECRARECTAR	BITTOCATTO	CCOGGGCCGG	DECEMBER	COCCOCCTO	TETOPE
	1561 1	1570	1580	1594593										
	TCTRCA	ictge tarb	CCCAMARA CCCAMARA CCCAMARA	STARGETG										

Fig. 3

	1	10	20	30	. 40	50	60	70	80	90	100	110	120	130
J04353 IPV31_11 Consensus	HILLIE	. 1	aci di Garit	acineigie	HET HEEDENE	CTGTCCCOGTG CTGTCCCOGTG	TETRANCIT	AT THE COURT	ATGARINTGT	GULULUMEL	ABCATRI BIT	N TENERCOCCI	PROTOCTOCCE	TECTTO
	131	140	150	160	170	180	130	200	210	220	230	240	250	260
J04353 HPV31_L1 Conscnaus	CRETT CRETT	GGCCATCCA GGCCATCCA	ATTOTICCE TATTOTICCE TATTOTICCE	TRECTANATE TRECTANATE TRECTANATE	TORCENTEC TORCENTEC TORCENTEC	TRANSPIRATA TOROGRAFIA TRANSPIRATA	TIGINCOR TIGINCOR TIGIRCOR	NGETOTORES NGSTETORES RESTOTORES	ATTRONATAT ATTRONATAT ATTRONATAT	RSGETATTTA RGGSTATTTR RGGSTATTTR	65611C61111 65611C61111	NCCOGNICCO NCCOGNICCO NCCOGNICCO		ATTICE ATTICE ATTICE
	261	270	200	290	300	310	320	330	340	350	360	370	380	330
J04353 HPV31_L1 Consensus	15511	LHICKITTU	HORICCIG		THEFT	GCCTGTGTTGG GCCTGTGTTGG GCCTGTGTTGG	TTRESERT	nem cerens	COCCEDITION	CTCYOCCTOY	TOCTCCTCGT	PROTENTAN	1100017700	COCCO
•	391	400	410	420	430	440 .	450	450	470	480	490	500	510	520
JD4353 HPV31_L1 Consensus	ET MITTER		اشتكلنا الالا	COLL HAS	CIMIRRI	GGENATETUTA GGENATETUTA GGENATETUTA	TERRITARA	TOTARREDAR	COCOCCICIC	RTTRETTEST	TECHNOLOGIC	rtarteeneac	PRITCECTO	MOCETO
	521	530	540	550	560	570	580	590	600	610	620	639	640	650
J04353 RPV31_L1 Consensus	GICCI	TGTBGTERO	BOIGCTOTIA	CCCCTEGTER	TTGTCCTCC	NYTHGROYTHA RYTHGRATTAN NYTHGRATTAN	ANGOT FERG	TTOTOGRASS	TEREPROPE	GTTCO POPOC	CETTTGGGGG	POTECO EYY YO	PETERNITORO	OCOCOC
	651	560	670	680	690	700	710	720	730	740	750	760	770	780
JO4353 HPY31_L1 Consensus	1111001	6110416110	.1116680011	TIGIANTICI	HITTGTFAR	TATCCAGATTA TATCCAGATTA TATCCAGATTA	TETTRORRIT	SETTECTERC	CCATATGGGG	RTOSSSTATE	T FTYTRTTTN	CITT OF THE OWNER	RATE IT YATRAS	BLECHT
	781	790	800	BIO	820	830	840	850	860	B70	B80	B90	500	910
JO4353 HPV31_L1 Consensus	11111	113411854117	666365611	66 I 689 I CS	TOTAL BOOK	TATOTOTATT CITATOTATOT CITATOTATOTT	nnocia Try	CRITCHNCAG	TTRETTYOUR	TORPACTOR	TOPTTTPCYO	roccinacteci	Treetesti	CTIFOC
	911	920	930	940	950	880	970	980	930	1000	1010	1020	1030	1040
J04353 HPV31_L1 Consensus	TITGER	COURSETTIF	MTARACCAT	DITEGRICE	INCGTECTOR	GEORGRENNTR GEORGRENNTR GEORGRENNTR	ATGGTATTT	CTTGGGGCAR	COGTEST	STIRTIGUES	TARRITHCCRCS	TOTAL TROOP	MIRICICICI	TICTCT
	1041	1050	1050	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
J04353 HPV31_L1 Consensus	TGCRA	TTGCARREN	TERTRETRE	ATTIANARGI	RETRUCTOR	ANCAGTATTT WILLAGTATTT BANGAGTATTT	RAGREATEG	TGBGGRANTTT	CHALLULARIA	TRIBITIES	STYNTECHNOS	TARCOTTATO	TERRESTRE	DICHEO
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
J04353 HPV31_L1 Consensus	TATAT	TENERGIAIS	NATECTECT	ATTIT GGING	RTTGGARTTI	TEGRITOREC TEGRITOREC TEGRITOREC	RCRECTEC	TCOSSTICTT	rgerrentaci	TRYMERTY	TOURTHORN	TELEPORT TREE	TOTCORRESON	LICCLE
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
JO4353 HPV31_L1 Consensus	CECRR	RAGE CERRAGA	MIGATECAT	TTRABBATTO	TGTATTTTGE	GOGGTTANTT GOGGTTANTT GOGGTTANTT	TARANGARRI	NGT FT TET GET	RESTTYBEST	TREAT TITCERCY	GGGTCGCAAA	TTTTTTTTTC	RECERCENTE	TREET
	1431	1440	1450	1460	1470	1480	1490	1500	151812					
J04353 HPV31_L1 Consensus	REGTER	CTDAATTTAA	RGCRGGTRR	BCGTRSTGCN	CCC TCNGC#1	CTRECRETRES CTRECRETRES CTRECRETRES	RECOGERRA	CETHORNERS	CTARRARA					

Fig. 4

	1	10	20	30	40	50	60	70	80	90	100	110 .	120	130
HC_001525 HPV11_L1 Consensus	ATGT ATGT ATGT	EGCEGECTRE EGCEGECTRE EGCEGECTRE	CENCRECAC CENCRECAC	ACTATATOTO ACTATATOTO ACTATATOTO	ETCETCECA ETCETCECA ETCETCECA	ACCCTETATO	CREGETTET CREGETTET CREGETTET	TECCHICGENTO TECCHICGENTO TECCHICGENTO	CETATETTAR CETATETTAR CETATETTAR	REGERECARE REGERECARE REGERECARE	OTATTTTBTC OTATTTTATC	TTGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TETRENCTO TETRENCTO TETRENCTO	TTECTE
	131	140	150	150	170	1B0	150	200	210	220	230	240	250	260
HPV11_L1 Consensus				MARINGT TR MARINGT TR MARINGT TR										
	261	270	280	230	300	310	320	330	340	350	350	370	380	390
HPV11_L1 Consensus	GTTT	ACCCCRETE		TRETATGEGE TRETATGEGE TRETATGEGE										
	391 	400	410	420	430	440	450	460	470	480	490	500	510	520
HPV11_11 Consensus				GATANTAGG GATANTAGG GATANTAGG										
	521	530	540	530	560	570	5B0	530	600	ET0	650	630	640	650
HPV11_L1 Consensus				GCCCCCCGTT GCCCCCCGTT										
	651	650	670	680	630	700	710	720	730	740	750	760	770	780
HC_001525 HPV11_L1 Consensus				CTGCARATAT CTGCARATAT CTGCARATAT										
	781	790	800	810	820	830	B40	850	860	870	880	690	200	910
KC_001525 KPV11_L1 Consensus				CC TENTENCO CC TENTENCO CC TENTENCO										
	911	920	530	940	950	550	970	980	990	1000	1010	1020	1030	1040
RPV11_L1 Consensus				RGGCTCAGGG RGGCTCAGGG RGGCTCAGGG										
	1041		1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
NC_001525 HPV11_L1 Consensus				TTRTRAGGRA TTRTRAGGRA TTRTRAGGRA										
	1171		1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1230	1300
				RACTTIGOTT RRCTTIGGTT ORCTTIGGTT										
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
NC_001525 HPV11_L1	NGCAT NGSNT			TTTGGGRGGT TTTGGGRGGT TTTGGGRGGT										
	1431		1450	1450	1470	1480	1490	1501503						
NC_001525 IPV11_11 Consensus	TREAD	ITOTBANGCO	CCCRGCTGT	STETIRGECET STETIRGECET STETIRGECET	CTRCRECCE	CORREGERR	RCG TRICCRRI	INCORREGER						

Fig. 5

	1 10	20	30	40	50	60	70	80	90	100	110	120	130
HC_001357 HPV18L1-0 HPV18L1-L	RISTSCETSTRI RISTSCETSTRI	NEREGGSTEETE RENEGGSTEETE	ATATTOCOT MINTOCOT	INCONTETNE	INCETETOTAL INCETETOTAL	LESCOCULLE.	THTORCCORC	ESCCCTECC RSCCCTECC	TETRENERSTI TETRENERSTI	THTTESTATI	CATEGINES CATEGINES	CATTOTIATI	GTESCC GTESCC
Consensus	ATETECCTETAT	RERESGUECTE	MIGITRENT	INCONTETNE.	.ACCTETGTO	rescontre	TATEGOCONO	.GCCCCTGCC	TETRICACRETI	ITETTECTATI	ACRT GGT NCA	COLLULATION	EIGECC
	131 140	150	150	170	100	190	200	210	220	230	240	250	260
HC_001357 HPV18L1-U HPV18L1-L	ALIBIALIBILI	THITTCCTRRGRE	RACGTRARCG	IGTTECETAT	TTTTTECRE	TEGCT TET	GECGECCTAG	TGACRATACC	THIRICITO	RECTEETTE	TETESCANDA	GTTGTBRATA!	EGATEN
Consensus	ATTRIBITATIO												
	261 270	2B0	290	300	310	320	330	340	350	360	370	380	330
HC_001357 HPV18L1-U HPV18L1-L Consensus	TTATGTENETCE TTATGTENETCE	CUCHRECHIRI	it ini can GC	GGCHGCTCTI	Kara Tan Trok	1611G01IW1	recentari i i	RESULTED	3006160100	2011 HINGS 102	BIHITCCIN	EGOT ITCTGC	THICCHIN
· ·	391 400	410	420	430	440	450	ara)	470	480	490	500	510	520
NC_001357	1	REFETECRETTE	CCTERCOOR!										<u></u> ĭ
HPV18L1-U HPV18L1-L	THTREASTRITT THTREAGTRITT	REEGTECHETT	ICC T GALCCERS	minerita	TTRECTGR	INCINCINTI	INTRATETTE	RARCACORCE	TIRGIGIES	CCTGTCCTG	ERETECIMAT	TEGECCG T CET	RECETT
Consensus	TOTRERSTOTT	DEGETECRETTE	ECTGACCCR	भाषभास	ATTERCET GAT	INCTRETATE	ETTABTC.TG	UNACACIANCE.	TTRETGTGG	CCTGTGCTGC	SAGYGGRANT	TESCCETEST	RECETT
	521 530	540	550	560	570	580	530	600	610	650	630	640	650
HC_001357 HPV18L1-U HPV18L1-L	TREGTETTEGEC	TTRETECCENT	CATTTTATAS	TRANT TASAT	TERCRETTERNS	ISTICCCATE	CECCACGTE	TRATETTICT	SAGGACGTTA	1	TCTBTRERT	TATTRECASAL	RCRGTT
Consensus	PRESTOTTEGEC												
	651 660	670	680	630	700	710	720	730	748	750	760	770	780
HC_001357 HPV1BL1-U HPV1BL1-L Consensus	ATETRITTIESS	CTGTGCCCCTGC	TATTEGGGA	CRCT666CTI	PARSGENE 1 SC	TTGTARATC	SCETCETTIA	TEREROGOGG	11760CCCCC	TTREARCTT	BRANCACAG	TTTTGGRAGN	GGTGAT
	781 790	800	810	B20	830	840	B50	860	870	880	890	900	910
KC_001357 KPV1BL1-U IPV1BL1-L Consensus	ATEGTREATACT	GCATATEGT GCC	CATGGACTTT	GTACATTEC	MGATACTARA	TETGREGTR	CATTEGATA	TTIGTCRETC	IATTTGTARA	ATCCTGATT	TTTRCARRY	STETGERGATO	ETTATO
	911 920	930	840	950	960	970	980	990	1000	1010	1020	1030	1040
NC_001357 HPV18L1-U HPV18L1-L Consensus	GGGAYYCCATGT	TTTTTTGCTTAC	GGCGTGRGC	GCTTTTTGC	TRGGCOTTTY T	GGRATRGAG	CAGGTACTAT	GESTERCRET	STECCTCAST	CTTATATAT.	TRANSGEACH	GGTATECCTG	TTCRCC
													•
	1041 1050	1060	1070	1080	1090	1100	1110 .	1120	1130	1140	1150	1150	1170
NC_001357 NPV18L1-U RPV1BL1-L Concensus	1041 1050 TGGCRGETETGT												
NPV18L1-U HPV1BL1-L	I												
HPV18L1-U HPV1BL1-L Concencus	TESCHECISTET	GINTICTCCCTC	1200	1210	1220	CCAGTIGTTI	1240	1250	1260	1270	1280	GETGGERTRAT	1300
IPV18L1-U HPV1BL1-L Concencus	1171 1180 1176 1180	1190 GTREATRICERCT	1200 TCCRRTRCC	1210	1220	1230	1240 CCTGTACCTG	1250 GGCRATATGA GGCRATATGA GGCRATATGA GGCRATATGA	1260 IGCTACCHANI IGCTACCHANI IGCTACCHANI	1270 TTANGCRETI	1280 ATRICAGACA ATRICAGACA ATRICAGACA	1290 TOTTGREGANT TOTTGREGANT	1300 TRIGATI
HPV18L1-U HPV18L1-L Concencus HC_001357 HPV18L1-U HPV18L1-L Consensus	1171 1180 11761TRETETE	1190 GTREATRICERCT 1320	1200 1200 TCCCRGTRCCS	1210 1210 1340	1220 INTERFECTOR 1230	1230 TRERERETETE CRETCHE CRETCHE 1350	1240 CCTGTACCTG CCTGTACCTG CCTGTACCTG	1250 GSCRATATOR GSCRATATOR GSCRATATOR GSCRATATOR 1300	1260 IGCTACCAAN IGCTACCAAN IGCTACCAAN IGCTACCAAN IGCTACCAAN 1390	1270 TTANGCRETI TTANGCRETI TTANGCRETI	1280 HTAGCAGACA HTAGCAGACA HTAGCAGACA 1410	1290 TOTTGREGAN TOTTGREGAN TOTTGREGAN	1300 TRICATI TRICATI TRICATI TRICATI
HPV18.1-U HPV18.1-L Concencus NC_001357 HPV18.1-U HPV18.1-U HPV18.1-U HPV18.1-U HPV18.1-U	1171 1180 1171 1180 1171 1180 1301 1310 160661110111	1190 GTREATACCACT	1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200 1200	1210 PATTTERCER 1340 PACTGERGE	1220 181010CTTCT 12350 1011010CTCCT	1230 FRENCHETTT	1240 CETGTACETG CETGTACETG CETGTACETG 1370 CETGTACATG	1250 GECHATATON GECHATATON GECHATATON GECHATATON GECHATATON GECHATATON GECHATATON LOGIOTITION CONGINITION CONGINITION	1260 IGCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACCARATICCTACARATICAT	1270 TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI	1280 HTAGCAGACA HTAGCAGACA HTAGCAGACA 1410 HTCCCCCCCCC	1290 TOTTGREGANT TOTTGREGANT TOTTGREGANT 1420 CONNECTNECTNE	1300 (ATGATT (
HPV18L1-L Concencus NC_001357 HPV18L1-L Consensus NC_001357 HPV18L1-L NC_001357 HPV18L1-L	1171 1180 1171 1180 1161TACTRITE 1301 1310 16CAGTITATTI	1190 GTREATRICERCT 1320 TTCREATTGTGTGTT TTCREATTGTGTGTT	1200 TECHNOTOGE 1200 TECHNOTHECE 1330 TECHNOTHECTTI	1210 ATTTERCRA 1340 FRACTIGERGHI MACTGERGHI MACTGERGHI	1220 IRIGIGETTET 1350 IGTIRIGICETTET ISTIRIGICETTET ISTIRIGICET	1230 FRENCHETTI CRESTETT CRESTETT 1350 FRISTITENTE	1240 CCTGTACCTG CCTGTACCTG CCTGTACCTG CCTGTACCTG 1370 STATGAATAG GTATGAATAG	1250 GGCRATATGA GGCRATATGA GGCRATATGA 1280 CAGTATTTA	1260 TGCTACCRARI TGCTACCRARI TGCTACCRARI 1390 ERGERTTGCRA	1270 TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI 1400 ECTTGGTGTT	1280 HTAGCAGACH HTAGCAGACH HTAGCAGACH 1410 FECCECCECC	1290 TOTTGREGARI TOTTGREGARI TOTTGREGARI 1420 CONNECTRETRE	1300 THICATT THICATT THICATT 1430 THICAT THICAT
NPY18.1-1 HPY18.1-1 HPY18.1-1 FY18.1-1 Consensus NC_001357 HPY18.1-1 HPY18.1-1 HPY18.1-1 Consensus	1171 1180 1171 1180 1171 1180 1161TACTRIC	1190 6TREATACCACT 1320 17CACTIGTGTG 17CAGTIGTGTGTT 17CAGTIGTGTGT	1200 TECRRETRECE 1330 TETRITRETTI TETRITRETTI	1210 HATTTERCERS 1340 HACTGERSSI HACTGERSSI 1470	1220 INTERCETTE 1350 INTERCETTE 1350 INTERCETTE INTERCETTE 1480	1230 FRERENGTETT CRETETT CRETETT 1350 FRITTICHTEE FRITTICHTEE FRITTICHTEE 1498	1240 CCTGTACCTG CTGTACGATTAG CTGTACGAT	1250 GGCRATATGA GGCRATATGA GGCRATATGA 1280 CAGTATTTA	1260 TGCTACCHAR TGCTACCHAR TGCTACCHAR TGCTACCHAR 1390 SHGGATTGGAT SHGGATTGGAT SHGGATTGGAT	1270 TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI 1400	1280 RITAGERGACH RITAGERGACH RITAGERGACH RITAGERGACH 1410 RECCECCECC RECCCCCCCCCCCCCCCCCCCCCCCCC	1280 TOTTGREGAN TOTTGREGAN TOTTGREGAN TOTTGREGAN 1420 ECHRETRETRE	1300 (HTGATT (
NPY18.1-1 HPV181.1-1 HPV181.1-1 Consensus NC_001357 HPV181.1-1 Consensus NC_001357 HPV181.1-1 Consensus	1171 1180 1171 1180 1171 1180 1301 1310 160067110711 160067110711 1431 1440 160067110717	1190 GTREATHCERCT 1320 TTCRETTGTGTGTTTCRETTGTGTGTTTCRETTGTGTGTTTTCRETTGTGTTTTTCRETTGTTTTTTTTTT	1200 TECRAGISCS 1200 TECRAGISCS 1330 TECRATICETTI TECRATICETTI 1450	1210 HITTIBRICAN 1340 HACTGERGNI HACTGERSNI 1470 HACTGERST	1220 INTOTECTION 1.350 GETTATETECT 1.480 INGENTACETECT	1230 FIGURE SETEM CRESCRICTI CRESCRICTI CRESCRICTI 1350 FIGURE SETEM FIGURE SETEM FIGURE SETEM 1440 FIGURE SETEM FIGURE SE	1240 CCTGTACCTG CCTGTACCTG CCTGTACCTG 1370 GTATGATTGA	1250 GECHATATOR GECHATATOR GECHATATOR GECHATATOR GECHATATOR GECHATATOR CRETOTITATOR	1260 TGCTACCRAN TGCTACCRAN TGCTACCRAN TGCTACCRAN 1390 SINGGATTGGAI SINGGATTGGAI 1520	1270 TTARGEASTI TTARGEASTI TTARGEASTI TTARGEASTI TTARGEASTI 1400 ECTTGGTGT ECTTGGTGT 1530	1280 THE CONTROL OF	1280 TOTTORGERN TOTTORGERN TOTTORGERN 1420 ECURCTRETER ECURCTRETER 1550	1300 (HTGATT (
NPY18.1-U HPY181.1-U HPY181.1-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U HPY18.11-U	1171 1180 1171 1180 1171 1180 1161TACTRIC	1190 6TRENTEDERCT 1320 17CRESTICTORY 17CRESTICTORY 17CRESTICTORY 1450 17TRESPORT	1200 1200 1200 1230 1231 1231 12450 1250 12450 12450 12450 12450 12450 12450 12450 12450 12450 12450 1	1210 1340 1340 1340 1340 1340 1347 1470 16CCTOTCASS	1220 INTERPRETATION 1350 INTERPRETATION 1450 INSENTING THE TRANSPORTED THE T	1230 PREMERCICAL CASTETY CASTETY 1350 POTENTICATA CASTETY TABLE 1450 ACCEGETGAR RECEGETGAR RECEGETGAR RECEGETGAR	1240 CETGTACETG CETGTACETG CETGTACETG 1370 CITTGANTAG GTTGANTAG	1250 CECHATATOR 1330 CONTRITTION CONTRIT	1260 IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCATICGAI	1270 TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI 1400 ECTTEGGTGT ECTTGGTGTT 1530 TTGGRATGTGT	1280 RITIGERAGE RITIGERAGE RITIGERAGE RITIGERAGE 1410 RECCCCCCCC 1540 RITITERAGE	1289 TOTTORGORNI TOTTORGORNI TOTTORGORNI TOTTORGORNI 1420 CERROTRETRETRETRETRETRETRETRETRETRETRETRETRET	1300 PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT
NPY18.1-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1 HPV1811-1	1171 1180 1171 1180 1171 1180 1171 1180 1301 1310 16001110111 16001110111 1431 1440 16001101011	1190 6TRENTEDERCT 1320 17CRESTICTORY 17CRESTICTORY 17CRESTICTORY 1450 17TRESPORT	1200 1200 1200 1230 1231 1231 12450 1250 12450 12450 12450 12450 12450 12450 12450 12450 12450 12450 1	1210 1340 1340 1340 1340 1340 1347 1470 16CCTOTCASS	1220 INTERPRETATION 1350 INTERPRETATION 1450 INSENTING THE TRANSPORTED THE T	1230 PREMERCICAL CASTETY CASTETY 1350 POTENTICATA CASTETY TABLE 1450 ACCEGETGAR RECEGETGAR RECEGETGAR RECEGETGAR	1240 CETGTACETG CETGTACETG CETGTACETG 1370 CITTGANTAG GTTGANTAG	1250 CECHATATOR 1330 CONTRITTION CONTRIT	1260 IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCTACCHANI IGCATICGAI	1270 TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI TTARGERSTI 1400 ECTTEGGTGT ECTTGGTGTT 1530 TTGGRATGTGT	1280 RITIGERAGE RITIGERAGE RITIGERAGE RITIGERAGE 1410 RECCCCCCCC 1540 RITITERAGE	1289 TOTTORGORNI TOTTORGORNI TOTTORGORNI TOTTORGORNI 1420 CERROTRETRETRETRETRETRETRETRETRETRETRETRETRET	1300 PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT PATGATT
IPVIBL-1-L Consences NC_001357 HPVIBL-1-L Consences NC_001357 HPVIBL-1-L Consensus NC_001357 HPVIBL-1-L Consensus NC_001357 HPVIBL-1-L Consensus NC_001357 HPVIBL-1-L Consensus	1371 1160 1716TTRETRICT 1303 1310 16CRETTRITTI 16CRETTRITTI 1431 1440 GENTRETRITTI 1431 1440 GENTRETRITTI 6GENTRETRITTI	1190 GTHSHTACCACT 1320 TTCHATTGTGTGT TTCHGTTGTGTGTTTCTGTGTTTTCTGTGTTTCTGTGTTTTTTT	1200 1200 TCCCRRETECCE 1330 TCCCRRETECCE 1330 TCCTRTTRCTTT TCTTTTCTTT TCTTTCTTTT TCTTTCTT	1210 1210 1340 1340 1340 1340 1340 1340 1340 13	1220 1810TOCTIC 1350 GTTATGTCCT GTTATGTCCT 14680TGCTGT 18680TGCTGT	1230 FRENCHICTE CRETCTI CRETCT CRETCT CRETCT CRETCT CRETCT CR	1240 CCCGTACCTG CCCGTACCTG CCCGTACCTG CCTGTACCTG 1370 CTATGANTAG CTATGANTAG TATGANTAG	1250 COCCRETATOR 1380 COCCRETATOR COCCRETATOR COCCRETATOR CECCRETATOR CECCRETATOR CECCRETATOR CECCRETATOR CECCRETATOR CECCRETATOR 1640	1260 1260 1GCTACCARA: 1GCTACCARA: 1390 1390 1390 1310	1270 TTARGCASTI TTARGC	1280 ATAGGAGACA ATAGGAGACA ATAGGAGACA 1410 FECCECCECC FECCECCCCC ATAGGAGACA ATAGGAGACA ATAGAGAGACA ATAGAGACA ATAGAGAGACA ATAGAGACA ATAGAGACA ATAGAGAGACA ATAGAGACACA ATAGAGACACA ATAGAGACACA ATAGAGACACA ATAGAGACACA ATAGAGACACACACACACACACACACACACACACACACAC	1290 10TTGREGATI 16TTGREGATI 16TTGREGATI 16TTGREGATI 16TTGREGATI 16TTGREGATI 16TTGREGATI 16TTGREGATI 1420 CERRETRETATE CERRETRETRETRETRETATE CERRETRETATE CERRETRETRETRETRETRETRETRETRETRETRETRETRE	1300 (RTGRTT) (RTGRTT
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Fig. 6

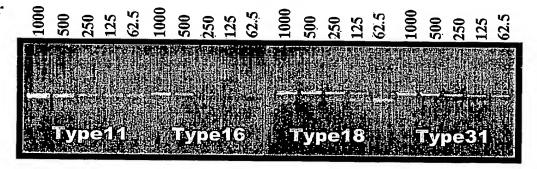
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Consensus	NeCCC 651	FT REGLET	670	E80	.t	ITELGATGA 700	acaGRARat.	ct.gTeg.tal 720	.gc.ggtaaTi 730	ctest.asGA	tooTAGgGa.	oaTgTatcta	Tegattrea	loCRaffC
HPV11-L1 HPV16-L1 HPV31-L1 HPV18-L1 Consensus	CCRSC ACRAT ACRAC ACRAC	TATETRIG TGTGTTIA TGTGTTTA	OTGGGCTGTGC OTTGGTTGCRA OTTGGTTGCRA OTGGGCTGTGC OTGGGCTGTGC	TCCRCCGTTR RCCRCCTRTR RCCRCCTRTT CCCTGCTRTT	GGTGRACAT GGGGRACAC GGRGRGCAT GGGGRACAC	regestanes regescanne regestanes	GTACACRATG GATCCCCATG GTAGTCCTTG GCACTGCTTG	TTCARATACCT TRCCONTGTTO TAGTARCARTO	CTGTRCARA COGTRATCO CTATTRCCCO	TOTTROTOOR TOTTROTOOT TOTTROORS	CCRCCATTRG CCTCCATTRG CCCCCTTTRG	RETTRATORS RRTTRARARS RRCTTRANSS	CRCRGTTRT1	CREGAT
HPV11-L1 HPV16-L1 HPV31-L1 HPV18-L1 Consensus	781 I GGGGN GGTGN GGGGN GGTGN	790 COTTGETTG TRIGGTTG TRIGGTRG TRIGGTRG	BOD PYRCRESCTTT NYRCTUSCTTT NYRCRESCTTT NYRCTUSNYRT NY	810 GGTGCTNYGR GGTGCTRYGG GGRGCTRYGG GGTGCCRYGG	829 ATTTI CREE BCTT CCTRE BTTT BCTEE BCTT DGTRE	830 CTTRCARRO CTTRCARGE CTTTRCARGE	840 CANTORATEGO TRACEBRARGIO CRETERRARGIO TRETENNATGIO	850 GOTGTTCCCCT GRAGTTCCACT HATGTTCCTTT GRAGTTACCATT	860 TGATATTIGT GGATATTIGT GGACATTIGT	970 GGARCYGTCT RCHICTRITTI ARTYCTRITTI CRGTCTRITTI	GERRATATEC GERRATATEC GERRATATEC GERRATATEC	850 TEATTATTIC CENTATOTI CENTATOTI	900 CARNTEGETO ARRATEGETO CARRATEGETO	910 CREACC CAGARC CTEREC
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Fig. 7

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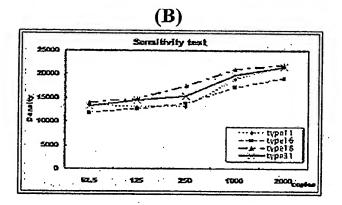


Fig. 8

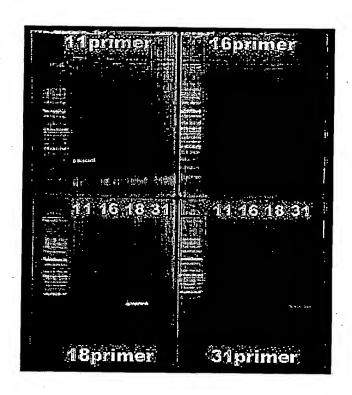
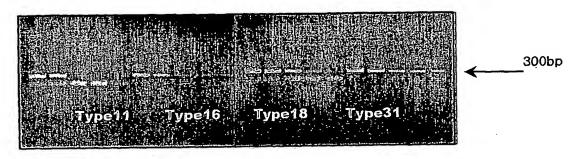


Fig. 9

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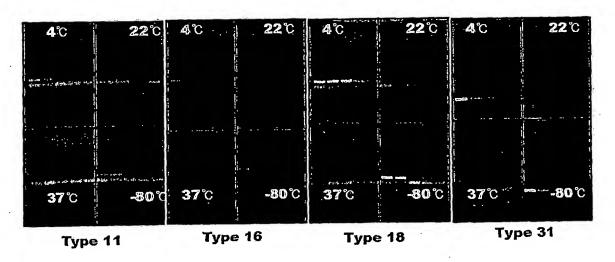
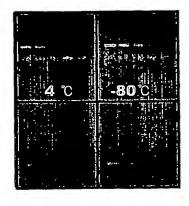


Fig. 10



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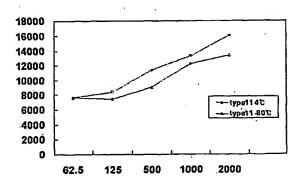
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Type11 & Type16



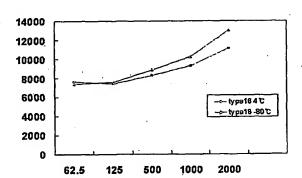
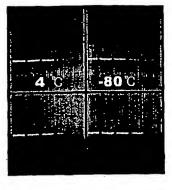


Fig. 11



Type18:4 ℃ & -80℃

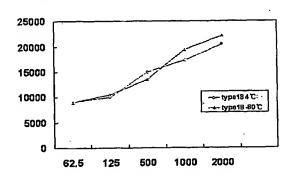
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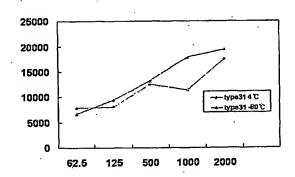
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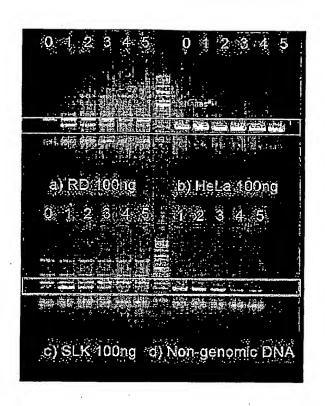
Type18 & Type31





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Fig. 12



WO 2006/098582

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PCT/KR2006/000915

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WO 2006/098582

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International application No. PCT/KR2006/000915

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC8 C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

NCBI PubMed, NCBI GenBank, eKIPASS "HPV, L1, high-risk probe, genotyping, etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Υ .	KR 2004/0036318 A (GENOMICTREE, INC., KR) 30 Apr. 2004 - see the whole document	1 - 8
Y	KR 2004/0078506 A (BIOMEDLAB CO., LTD., KR) 10 Sep. 2004 - see the whole document	1 - 8
Y	WO 2001/068915 A1 (BIOMEDLAB CO., LTD., KR) 20 Sep. 2001 - see the whole document	1 - 8
Y	WO 2003/027323 A1 (BIOMEDLAB CO., LTD., KR) 03 Apr. 2003 - see the whole document	1 - 8
Y	KR 2004/0083674 A (BIOCORE CO., LTD., KR) 06 Oct. 2004 - see the whole document	i - 8

Further documents are listed in the continuation of Box	۲C.
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See patent family annex.

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- "&" document member of the same patent family

Date of the actual completion of the international search

10 JULY 2006 (10.07.2006)

Date of mailing of the international search report

10 JULY 2006 (10.07.2006)

Name and mailing address of the ISA/KR



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Facsimile No. 82-42-472-7140

Authorized officer

SHIN, Kyeong A

Telephone No. 82-42-481-5589



International application No.

PCT/KR2006/000915

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet) 1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of: a. type of material a sequence listing table(s) related to the sequence listing b. format of material on paper in electronic form c. time of filing/furnishing contained in the international application as filed filed together with the international application in electronic form furnished subsequently to this Authority for the purposes of search In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished. 3. Additional comments:

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)				
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
1. Claims Nos.: 3-7 because they relate to subject matter not required to be searched by this Authority, namely:				
Although claims 3-7 relate to a method of diagnosing under Rule 39.1(iv), the search has been carried out and based on the alleged effects of the method.				
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:				
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows:				
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.				
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.				
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:				
• •				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:				
The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee. The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation. No protest accompanied the payment of additional search fees.				

Information on patent family members

International application No.
PCT/KR2006/000915

Patent document cited in search report	Publication date .	Patent family member(s)	Publication date
KR 2004/0036318 A	30/04/2004	None	
KR 2004/0078506 A	10/09/2004	None	
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· KR 2004/0083674 A	06/10/2004	None	.•